When exercised, dogs with cardiac denervation achieve a maximal heart rate in 90 to 100 seconds. An increase in the transmural atrial pressure, due to an increase in depth of respiration and in return of blood to the heart, might be expected to develop early in exercise and to be sustained throughout the period of work. In the experiments shown in Fig. 1 and 2, the maximal transmural atrial pressures were achieved in 15 seconds and 15 minutes, respectively, but the corresponding maxima in heart rates did not occur until 2½ and 45 minutes later, at which times the transmural pressures had declined considerably from their maximal values. Although the early increase in heart rate shown in Fig. 1 suggested that rate of change of pressure might play some part in the cardiac acceleration, at the maximal mean right atrial pressure shown in Fig. 2, there was a peak-to-trough excursion of 14 cm of H_2O in the right atrial pulse.

These data suggest that it is unlikely that either increases in absolute level of the transmural atrial pressure or in rate of change of this pressure are the factors responsible for the increase in heart rate during exercise in the dog with chronic cardiac denervation.

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Corticosterone in Lymphoma-Bearing Rats.* (28353)

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In lymphoma-bearing rats, glucocorticoids and potassium appear to have opposing effects upon the lethal and tumor-necrotizing properties of capsular polysaccharide from *Klebsiella pneumoniae*(1-3). Lethal and tumor-necrotizing effects of the lipid-free polysaccharide were inhibited by cortisol and corticosterone(2) but minute quantities of potassium, injected intravenously, augmented these biologic properties(3). However, glucocorticoids did not nullify the polysaccharide-potentiating action of potassium (3). Since altered biosynthesis of corticosterone, the major glucocorticoid of the rat, could have some bearing on these interrelationships, corticosterone in the plasma and urine was determined by 2 procedures in control and in lymphoma-bearing rats.

Materials and methods. Eight young, male Holtzman rats bearing subcutaneous Murphy-Sturm lymphomas(1) of 35-40 mm diameter (approximately 2 weeks following implantation) and 8 control rats, each 180-220 g body weight, were placed in individual cages and urine collected for 24 hours as 3 refrigerated 8-hour samples which were later pooled. During the collection period the diet of Purina Laboratory Chow, lettuce and water was withheld. Immediately following collection of urine, the animals were killed by neck clamp(4), blood withdrawn from the heart into heparinized syringe and plasma separated by centrifugation.

Urine samples, adjusted to pH 1.0 with HCl, and plasma samples were processed by procedures for extraction and purification of corticosterone as described by Moncloa, Peron and Dorfman(5). However, the final extracts were further purified by paper chromatography in toluene/propylene glycol(6), the zones containing corticosterone eluted with absolute ethanol, and the dry residues of these eluates partitioned between n-hep-

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TABLE I. Plasma Corticosterone Levels.

	μg per 100 ml	
	A*	B†
Tumor-bearing	83 ± 13‡	85 ± 4
Control	26 ± 8	22 ± 7

* Spectrophotometric determination in absolute ethanol.
† Fluorometric determination in conc H_2SO_4.
‡ Mean ± standard deviation.

tane and 70% aqueous ethanol. The dry residues of the aqueous ethanolic phases were redissolved in 0.05 ml absolute ethanol, 0.45 ml concentrated H_2SO_4 added to each sample in glass-stoppered containers, then kept 40 min in a dark place at 25°C. The corticosterone content was then determined in G. K. Turner model 110 fluorometer (primary filter, 490 mμ; secondary filter, 525 mμ). The remaining aliquots of ethanolic samples were examined for corticosterone content in Bausch & Lomb "Spectronic 505" recording spectrophotometer. The observed absorbance was corrected by Allen's formula(7). Four corresponding plasma extracts had to be pooled for the spectrophotometric determination but the relatively high corticosterone content of urine permitted spectrophotometric determination in a single sample. Urinary corticosterone was further identified in pooled urine extract from the 2 experimental groups by H_2SO_4 spectra. This procedure was not feasible for plasma extracts because of the small absolute amounts of corticosterone.

Results. In the present study the plasma and urinary corticosterone levels of control rats were comparable to those reported by others(8,9). Plasma concentration of corticosterone in lymphoma-bearing animals was almost 4 times higher than normal controls (Table I). Corticosterone excretion in urine per day was moderately increased in lymphoma-bearing rats (Table II) but due to their increased urine volume, the concentration of urinary corticosterone was reduced.

Corticosterone in both plasma and urine showed maximal absorption at 240 mμ in absolute ethanol. The spectra of urinary samples in concentrated H_2SO_4 were identical to spectrum of authentic corticosterone. Similar values for corticosterone were obtained by direct spectrophotometric and by fluorometric analysis. The fluorometric procedure was the more sensitive, permitting estimation of small absolute amounts of corticosterone in properly purified single samples from nonstressed control animals.

Comments. The elevation of plasma levels and increased urinary excretion of corticosterone in lymphoma-bearing rats indicated an augmented adrenal biosynthesis of this corticosteroid. In lymphoma-bearing rats the relatively greater increase in plasma levels of free corticosterone as compared to renal excretion could reflect a reduction in its urinary excretion or in an increase in its conjugation and degradation. Metabolites and glucuronide conjugates of corticosterone would not be detected in urine or plasma by the methods employed. In unpublished studies we found that lymphoma-bearing rats had an enhanced initial clearance from the plasma of intravenously injected free corticosterone-4-C^{14}, followed by elevated levels of its glucuronide conjugate, associated with a more rapid uptake by and disappearance from the tissues, including tumor, and accompanied by the prominent urinary excretion of the labeled steroid, its glucuronide conjugates and metabolites. It may be deduced, therefore, that biosynthesis of corticosterone was much greater than suggested by the augmented urinary excretion or plasma levels of free corticosterone.

TABLE II. Urinary Corticosterone Levels.

	Urinary vol per 24 hr	μg per 24 hr		μg per 100 ml	
		A*	B†	A	B
Tumor-bearing	6.8 ± .5‡	8.32 ± .63	9.06 ± 1.04	125 ± 8	134 ± 12
Controls	4.4 ± .6	7.00 ± .33	7.24 ± .45	160 ± 20	166 ± 27

* Spectrophotometric determination in absolute ethanol.
† Fluorometric determination in conc H_2SO_4.
‡ Mean ± standard deviation.

Mechanisms through which the rapidly growing tumor may act as a "stressor" are uncertain, but possibilities include a direct effect upon the adrenal or an enhanced secretion of ACTH without the usual "feedback" control. In normal rats intravenous administration of ACTH resulted in plasma corticosterone levels similar to those observed in lymphoma-bearing rats in the present study(8).

In the present study the increased urinary volume in tumor-bearing rats may have been attributable to the increased corticosterone levels. A similar increase in urinary volume has been reported in alloxan-diabetic rats with increased plasma corticosterone(9) and in adrenalectomized rats following administration of corticosteroids(10).

Corticosteroids as well as ACTH have been found to counter-act the tumor-necrotizing and lethal effects of lipid-free *K. pneumoniae* capsular polysaccharide in the intact as well as adrenalectomized rat(2). Presumably the augmented corticosterone levels in plasma of the lymphoma-bearing rat would offer some protection against the biologic effects of the bacterial polysaccharide, thereby suggesting one explanation for the inverse relationship of tumor-size and tumor-necrotizing effect of the polysaccharide. Another explanation would be that the prolonged elevation of plasma corticosterone in lymphoma-bearing rats led to the decreased mass of splenic, thymic and lymphoid tissue(1) and hence to reduced cellular or other mechanisms against the homologous lymphoma.

In the guinea pig, injection of *K. pneumoniae* polysaccharide resulted in marked transient elevation of 17-hydroxycorticosteroids(4). If the tumor-bearing rat responded to the polysaccharide in a similar manner, an additional increase in plasma or tissue levels of corticosterone would be anticipated. Such a postulated rise in corticosterone, however, would follow rather than precede many toxic manifestations thereby providing little protection against the toxic bacterial product(4).

Exogenous glucocorticoids did not block the potentiation by potassium of polysaccharide toxicity in lymphoma-bearing rats(3). The increased corticosterone levels in the lymphoma-bearing rats may have provided a maximal protection against potassium, exogenous corticosterone affording little additional protection.

Summary. Corticosterone in plasma and urine was determined by spectrofluorometry in sulfuric acid and by direct spectrophotometry in ethanol. Plasma levels and urinary excretion of corticosterone were elevated in rats with subcutaneous lymphomas. The high plasma concentration of corticosterone in these rats may help to explain the reduction in splenic, thymic and lymphoid tissue of lymphoma-bearing rats and certain interactions of exogenous steroids, quantity of lymphoma tissue and tumor-necrotizing effect of *K. pneumoniae* polysaccharide.

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